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TITLE: Integrated Device for Circulating Tumor Cell Capture, Characterization and Lens-Free Microscopy

PRINCIPAL INVESTIGATOR: Richard Cote, Ph.D.
Changhuei Yang, Ph.D.

CONTRACTING ORGANIZATION: University of Miami
Miami, FL 33136

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Richard Cote, Ph.D.
Changhuei Yang, Ph.D.

E-Mail: RCote@med.miami.edu

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14. ABSTRACT

In the past year, we have completed the task of adapting the cell capture technology for breast cancer CTC capture, identification and characterization. We have also completed the task of laser-scanning based imagining of samples and implemented the next phase project by successfully implementing a wide field-of-view and high-resolution holographic scanning microscope prototype. Our completed work puts us in an excellent position to finish remaining tasks in the no-cost extension period. The holographic scanning microscope work has been reviewed and will be published in Optics Letters and Optics Express.

15. SUBJECT TERMS
Holography; Holographic optical element; medical optics and biotechnology; Imaging systems; Scanning microscopy

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Table of Contents

Introduction:
This document reports on the progress during the second year (1/8/2010-31/7/2011) towards the work done collaboratively at University of Miami by the researchers led by Drs. Cote, Datar and Nadji.

Background:
The detection of circulating tumor cells (CTC) in the peripheral blood of breast cancer (BC) patients has been an area of interest and investigation over the past 25 years. For with metastatic breast cancer, CTC may indicate high metastatic potential and increased morbidity, and the ability to monitor CTC could provide a real-time assessment of therapeutic efficacy. The development of a cost-effective and high-throughput CTC analysis system would revolutionize the field of CTC detection, prognosis, and therapeutic response monitoring and target development. We are in the early stages of developing a device that could revolutionize the capture of CTC from the blood of patients with breast cancer cost effectively through use of a parylene microfilter-based CTC capture device (MFCD). The technology is based on filtering blood through a finely engineered parylene membrane microfilter, where the larger CTC (15-25 μm) are preferentially retained on the membrane while typical blood cells (2-8 μm) flow through. Our collaborators (Yang et al, Caltech) are developing a novel holographic scanning microscope that can image and analyze tumor cells at a high throughput rate. In this current proposal, we aim to integrate our novel CTC capture microfilter system with the holographic scanning microscope to permit harvesting and analysis of CTC at high resolution, low cost and high throughput rate. Studies in Aims 1 and a part of Aim 2 are to be conducted at University of Miami.

Specific Aims of the Proposal:
Aim 1. Adapt the MFCD technology for breast cancer CTC capture, identification and characterization. (Studies to be conducted at University of Miami)

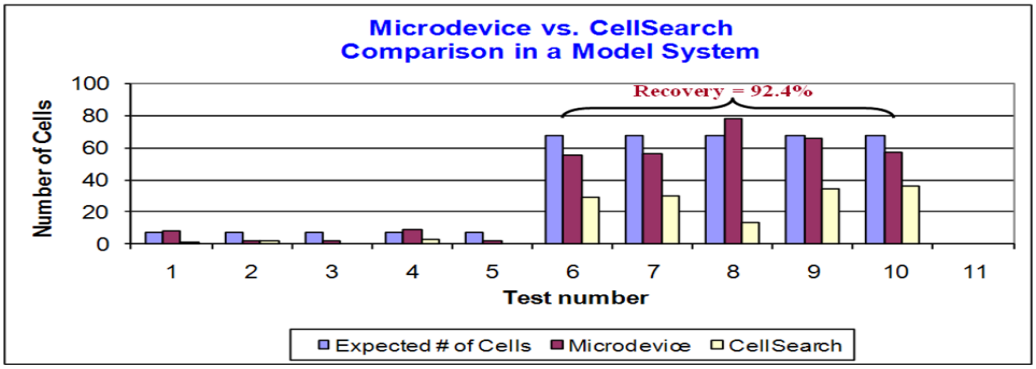
Aim 2. Fully evaluate the microscopy imaging needs associated with using MFCD technology for CTC capture, identification and characterization. (Studies to be conducted jointly at California Institute of Technology and University of Miami)

Aim 3. Implement a wide field-of-view (FOV) and high-resolution microscope system based on holographic scanning. (Studies to be conducted at California Institute of Technology)

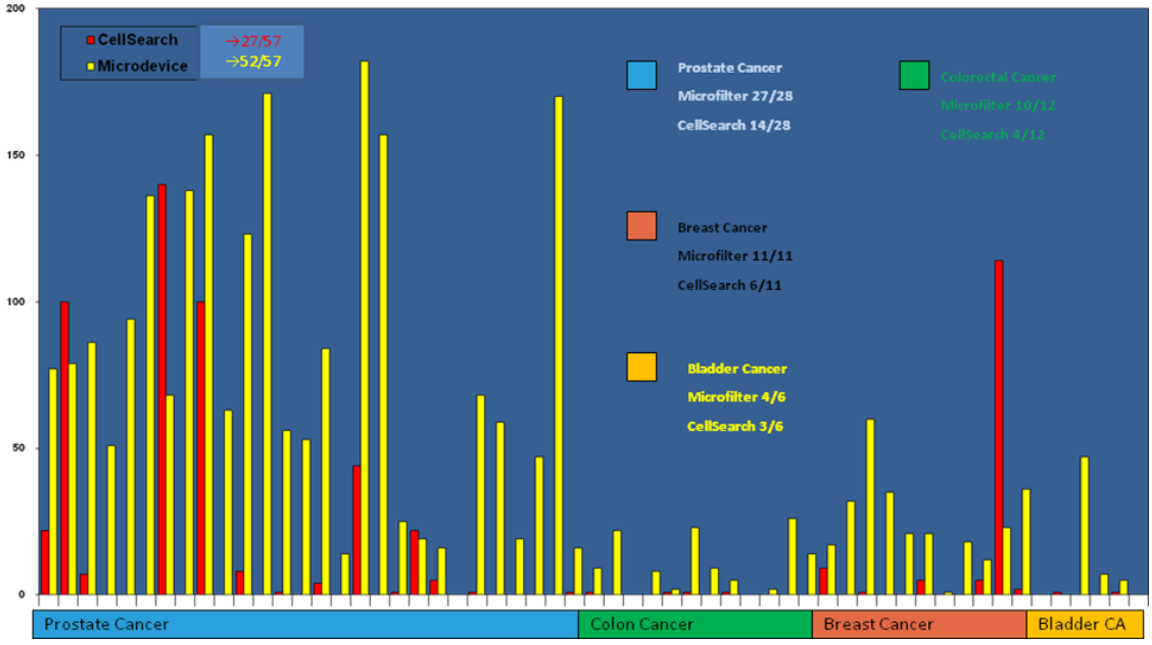
Aim 4. Combine two components to create a high throughput CTC analysis system. Develop software for identifying CTCs from debris. (Studies to be conducted at California Institute of Technology)

Key Research Accomplishments: (Bulleted list of key research accomplishments emanating from this research)

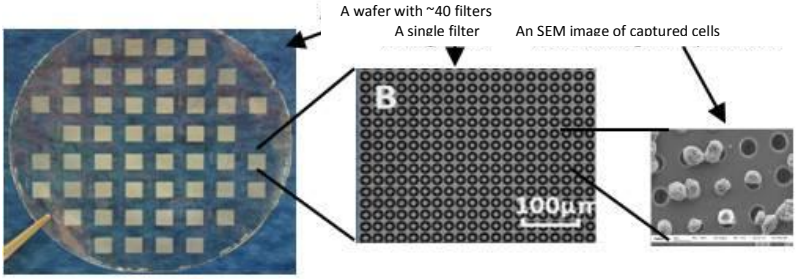
- MFDC showed excellent yield with tumor cells seeded in 7.5 ml of blood captured with >90% efficiency, and a high throughput (7.5 ml of blood filtered in <5 minutes, 20 ml of blood in <10 minutes) (See figure at right).

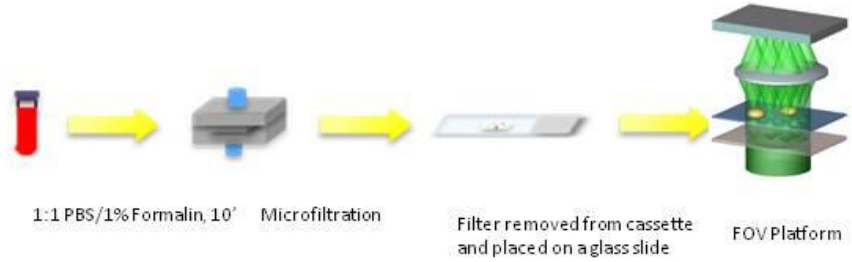


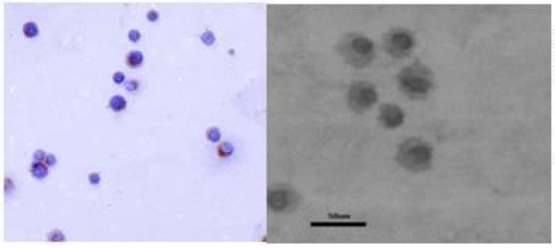
- This technology of CTC capture is shown to be substantially superior to commercially available FDA-approved immunomagnetic separation in blood from patients with cancer (Lin et al, Clin Cancer Res., 2010) (See figure at right). We have thus completed the task pertaining to Aim 1 of the proposed studies of achieving tumor cell capture through parylene-based microfiltration technology for breast cancer.



- Simultaneously, in association with our collaborator Dr. Yang (Caltech), we successfully demonstrated holographic recording technique which is highly stable and easy to scale (Aims 2 and 3). To achieve these Aims, five slides were prepared with cultured MDA-MB-231 Breast Cancer Cells and processed as follows: 1) Stained for Cytokeratin (CK) using chromogenic staining with DAB 2) CK Fluorescent Staining with Alexa 488 3) CK/CD45 Double Staining using Alexa 488/Alexa 594 combination 4) Negative Control Staining (slide incubated with no primary antibody but with only the secondary antibodies) 5) Unstained slide. All cell preparations were acetone fixed. The slides were sent at room temperature, cover-slipped. Each slide had low, medium and high cell density spots to help visualization by holographic scanning microscope. All slides were visualized and photographed at University of Miami before being sent, these records were compared with the images using holographic scanning microscope sent subsequently by Dr. Yang. The figure above shows representative comparative images of chromogenically stained T24 bladder







Left: Brightfield image Hematoxylin-Eosin stained Cytospin slides from T24 cells Right: Wide field-of-view microscope image of the same slide

cancer cells observed in brightfield microscope and the corresponding WFOV image. The experiments with imaging of fluorescently labeled cells are ongoing.

Reportable Outcomes: Provide a list of reportable outcomes that have resulted from this research to include:

- Our CTC microfilter applicability in cancer patient samples has been documented in a publication in Clinical Cancer Research.[1]
- We recently presented our data at the DOD Era of Hope conference in Orlando (August 2-5, 2011) and are preparing a manuscript for publication specific to the collaborative study in the coming months.
- No patents or licenses have yet resulted at University of Miami from this work, although our Caltech collaborator has filed for a patent.
- We have submitted an NIH R21 proposal for funding based on work supported by this award.
- Employment of a technician Jorge Torres-Munoz is supported at 40% by this award.

Conclusion: Our proof-of-concept prototype demonstrates that the proposed technology is feasible and represents an effective way to process CTCs. We are currently working to complete the project and have requested a no-cost extension to complete these studies.

Progress:

In our work so far, we have completed studies for Aim 1, and 2. Our microfluidics capture device (MFCD) has been shown to be capable of capturing CTC with extraordinarily high efficiency, both in model systems and patient blood samples. MFDC showed excellent yield with tumor cells seeded in 7.5 ml of blood captured with >90% efficiency, and a high throughput (7.5 ml of blood filtered in <5 minutes, 20 ml of blood in <10 minutes). This technology of CTC capture is shown to be substantially superior to commercially available FDA-approved immunomagnetic separation in blood from patients with cancer.[1] *We have thus completed the task pertaining to Aim 1 of the proposed studies of achieving tumor cell capture through parylene-based microfiltration technology for breast canc*er. *Simultaneously*, i*n association with our collaborator Dr. Yang (Caltech), we successfully demonstrated holographic recording technique which is highly stable and easy to scale (Aims 2 and 3).* Specifically, Dr. Yang's team has created a prototype of the target microscope system and employed it to image microscope slides. This prototype consists of an Excelsior-532-200-CDRH laser (wavelength = 532 nm) as the light source, a simple Thorlabs MAP10100100-A lens pair to project the light transmission from the sample onto a 2D sensor. We have shown preliminary transmission images of cultured tumor cells with this prototype microscope where the cells are captured on microfilter. Together with us, Dr. Yang's laboratory determined that CTC image identification requires 0.6 micron resolution or better. We are now building a fluorescence imaging prototype, which will put us in a good position to completing Aim 3 and begin with Aim 4 studies.

Ultimately, we will develop a lens-free holographic scanning, high-resolution and wide field of vision (FOV) microscope method that is well suited for analysis of CTC captured on the membrane, a technology that will allow frequent and regular assessment of cancer monitoring through CTC analysis. This platform will have an easy access to microscopy images of CTCs for the pathologist through generation of a montage of the identified CTCs. We will also develop the device to accommodate both classic chromogenic and fluorescence immunohistochemical procedures.

References:

Lin HK, Zheng S, Williams AJ, Balic M, Groshen S, Scher HI, Fleisher M, Stadler W, Datar RH, Tai YC, Cote RJ. Portable Filter-Based Microdevice for Detection and Characterization of Circulating Tumor Cells. Clin Cancer Res October 15, 2010 16; 5011

Appendices:

None